Effects of Union Mild Etch, a Newly Recognized Disorder, on Almond Scions Growing on Marianna 2624 Rootstock

J. K. Uyemoto, USDA-ARS, University of California, Davis 95616; **J. H. Connell,** University of California Cooperative Extension, Oroville 95965; and **C. A. Greer,** Department of Plant Pathology, University of California, Davis 95616

ABSTRACT

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Symptoms of union mild etch (UME) disease appeared on young trees of almond (*Prunus dulcis*) and consisted of light green to yellow drooped leaves that detached prematurely from current-season shoots otherwise exhibiting normal growth and development. Concomitantly, a mild etching was observed in the woody cylinder at the junction of the almond scions and the plum rootstock, Marianna 2624 (*P. cerasifera* × *P. munsoniana*). Affected trees were diagnosed in several orchards; one orchard had a 75% incidence and some trees died the same year they were diagnosed. However, many trees appeared to recover, with partial or complete lack of symptoms. Transmission attempts by grafting bud chips and bark patches to healthy almond/Marianna 2624 trees proved unsuccessful. Similarly, assays for viral, phytoplasma, and root rot pathogens were negative. The causal agent of UME is currently unknown. Measurements of tree trunks and nut harvests over four seasons showed that UME-affected Mission trees had significantly lower yields and less vegetative growth than healthy trees.

Additional keywords: tree size, union disorder, yield efficiency

Although the predominant rootstock for almonds continues to be peach (*Prunus persica* (L.) Batsch), there are increased demands for the plum rootstock, Marianna 2624 (*P. cerasifera* Ehrh. × *P. munsoniana* W. Wight & Hedr.) because more marginal orchard sites are being planted. Marianna 2624 has moderate resistance to *Armillaria* and *Phytophthora* spp. and *Meloidogyne* nematodes, and tolerates heavy, wet soils. Beginning in 1988, several young almond orchards established on Marianna 2624 were found to contain trees exhibiting premature leaf senescence and defoliation. Additionally, affected trees had shallow pits and grooves at the graft union and a few of the trees collapsed and died. These symptoms in almond trees have not been described previously.

To develop further information on union mild etch (UME), an investigation was initiated in 1990. Results of orchard surveys and field and laboratory assays, and effects of UME on tree performance, are reported.

Corresponding author: J. K. Uyemoto

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MATERIALS AND METHODS

Orchard surveys. During July through September, several young (first- to seventh-leaf trees) almond orchards located in the Sacramento and San Joaquin valleys of California were surveyed for trees with UME symptoms, i.e., light green to yellow drooped leaves, premature defoliation, and mild etching at the union. The unions were examined by removing the bark with a sharp knife and examining the woody cylinder. Some of the orchards were surveyed annually for 5 years. First-leaf is the same year of planting.

Graft transmission trials. Shoots and tree trunk sections that included the graft unions were collected from UME-affected and healthy trees of Carmel, Mission, and Peerless almonds located in three orchards.

In one series, seven and five collections of diseased scions and rootstocks, respectively, and two healthy Peerless scions were used in transmission tests to second-leaf Peerless almond indicators. Three healthy Peerless tree per collection were grafted; each with three to 10 scion buds inserted beneath bark flaps or with three bark patches of scion trunks or rootstocks directly grafted onto the cambium of the woody cylinder of the scion or rootstock portions of test trees. In another series, 19 healthy Carmel almond indicators were similarly grafted with bark patches from collections of diseased Carmel and Mission, and healthy Mission. All indicator trees were propagated on Marianna 2624 plum rootstocks.

Other assays. Succulent leaves and shoots collected from diseased and healthy trees were extracted in buffer and the extracts tested in enzyme-linked immunosorbent assay microtiter plates for prune dwarf virus and Prunus necrotic ringspot virus (4). Also, leaf mid-rib and petiole tissues or cambial scrapings from bark (scion and rootstock samples prepared separately) were extracted and purified for the viral replicative form, double-stranded RNA (dsRNA) (the presence of which can indicate virus infections), and analyzed by acrylamide gel electrophoresis (6).

For phytoplasma assays, tissues were extracted for total nucleic acids by the CTAB (cetyltrimethylammonium bromide) method (1). Purified extracts were subjected to polymerase chain reaction with primer sets derived from 16S (2) and 23S ribosomal RNA genes of phytoplasmas (polymerase chain reaction assays done by B. C. Kirkpatrick, University of California, Davis).

For fungal isolations, collections of roots from diseased and healthy trees and soils beneath sampled trees were assayed by plating root pieces onto potato dextrose agar and PARP selective medium (3). Soil collections were baited with pear fruit to detect *Phytophthora* spp. All fungal isolation work was supervised by S. M. Mircetich, USDA-ARS, University of California, Davis.

Trunk diameter and nut yield. Measurements of trunk diameters and nut harvests began in 1991 and continued through the 1994 season in a commercial Mission almond orchard established in 1988. Based on 1990 surveys, 40 UME-affected and 20 healthy Mission almond trees were selected for the study and assigned to paired plots that included two UME-affected trees and one healthy tree. In January 1991, one randomly selected UME-affected tree in each replication was pruned to remove half of the vegetative growth by thinning primary limbs. The remaining diseased and healthy trees were unpruned. It is noteworthy that a few diseased trees had died during the 1990 season, while most of them recovered and remained in remission for the duration of the experiment.

Trunk diameters were determined in October prior to leaf fall with annual measurements taken approximately 0.45 m

above the soil surface; the measured trunk area was marked with latex paint as a reference point. Harvest in September 1991 was accomplished by knocking scaffold branches with a padded mallet; nuts were collected and fresh weights recorded. Whole nuts were dried and the net kernel weights were determined. In 1993 and 1994, a mechanical trunk shaker was used. Nuts and debris were raked into a container and their weights recorded. Then, an approximately 1.8-kg subsample was removed, the in-shell almonds separated out and dried, and the kernels shelled out and weighed. Yield efficiencies were calculated as net kernel weights divided by the trunk cross-sectional areas.

To determine nut set, four groups of 100 flowers were counted on branches marked with flagging tape on each of five UME-affected and five healthy trees during February 1994. Two additional groups of 100-flower counts were made on flagged branches on an additional five UME-affected and five healthy trees in March. On 1 July, the numbers of developing nuts were recorded and the flower and nut counts per tree per treatment were averaged. The 10 UME-affected and 10 healthy trees were treated as single-tree replicates.

Statistical analysis. Data on trunk growth, nut yield, and yield efficiency were subjected to analysis of variance with PROC GLM (SAS Institute, Cary, NC) for the unbalanced data sets because midway in the trial three diseased trees were mistakenly used as healthy controls and were dropped from the experiment. Data for nut set were analyzed with PROC ANOVA. Means separation was performed with Fisher's least significant difference test (LSD) on data sets that resulted in a significant F value ($P = 0.05$).

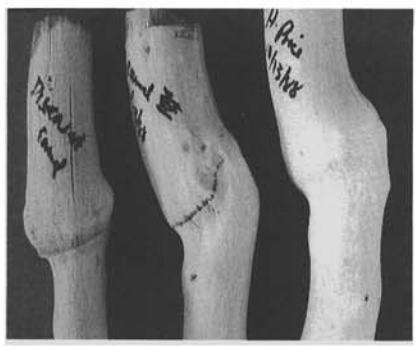

Fig. 1. A composite of graft unions illustrating (i) symptoms of union mild etch (UME, left), (ii) deep pits of almond brown line decline (ABLD, center), and (iii) healthy trunks (right), from trees of almond scions propagated on Marianna 2624 rootstocks.

RESULTS AND DISCUSSION

UME-affected trees produced apparently normal growth at budbreak to April. Thereafter, foliage on shoots of affected trees turned light green to yellow and drooped. By mid-summer, UME-affected trees began to defoliate, while healthy trees retained their leaves. Canopy symptoms, strongly evident on second- and third-leaf trees, also were observed in first-leaf plantings. Typically, as the trees matured, UME-associated symptoms were not observed again on previously symptomatic trees beyond fourth-leaf growth stage.

During summer to fall, an examination of the woody cylinder of diseased trees revealed a mild etch symptom along the junction of the scion and rootstock (Fig. 1); union symptoms were prominent on Butte, Carmel, and Price, but less so on the other diseased cultivars. In contrast, the unions of healthy trees were smooth and unetched.

As mentioned earlier, some UME-affected trees died in the same season disease symptoms first appeared; incidence of dead trees in an orchard was usually less than 3%. In one orchard, however, where nearly 30% of trees died (examination of these trees showed necrotic roots), all symptomatic trees were removed. At this time, it is not known if UME alone killed the trees.

Incidence of UME symptoms varied in orchards surveyed as follows: In one orchard of cv. Aldrich, incidence was 30%; in two orchards of Butte, incidence was 32 and 75%; in seven orchards of Carmel, incidence was 0, 15, 19, 21, 23, 60, and 68%; in three orchards of Mission, incidence was 13, 20, and 68%; in two orchards of Peerless, incidence was 1 and 49%; and in six orchards of Price, incidence in four was 0% and in two was 1%. Also, in four diseased orchards, alternate rows planted to either Padre or Sonora cultivars were healthy.

Although we had not assayed directly for a phytopathogenic bacterium, none of the other assays, including graft-inoculations, had associated UME with a biological agent. The grafted trees appeared healthy after three growing seasons. A few unions of the test trees were examined and these were normal. Also, assays performed for two ilarviruses, viral dsRNAs, phytoplasmas, and soilborne fungal pathogens were negative. Reference positive controls were included in these assays.

In the Mission almond orchard, trunk measurements over a 4-year period showed that UME-affected trees grew significantly less ($P < 0.05$) than healthy trees (Table 1). Trunk size of pruned and unpruned UME-affected trees was not significantly different ($P > 0.05$) for 3 years, but there was a significant difference in the means in the fourth year, with the pruned trees being larger. Statistical differences ($P < 0.05$) were found also in kernel yields (kg per tree) between healthy and diseased trees; however, pruned and unpruned diseased trees were not significantly different (Table 1). Differences ($P < 0.05$) were also noted in yield efficiency (gm of kernels per cm^2) in the first two harvests that varied among the treatments, but no significant differences were found by the third harvest (Table 1).

Percentage of nut set was 14.45 and 15.88, respectively, for UME-affected and healthy trees, and these values were not significantly different ($P < 0.05$).

The apparent lack of association of UME with a biotic agent suggests that UME might be genetic in origin. However, additional research must be done before accepting such a conclusion. Even so, occurrence of UME-affected trees in first-leaf orchards indicates that propagation of affected scion buds was likely involved. Progeny tests to determine the propagative nature of UME and investigations on disease etiology are being continued. Irrespective of UME etiology, we have demonstrated that the disorder reduces tree performance by reducing trunk diameter 15 to 20% and kernel weight 30 to 33% (Table 1). Moreover, the reduced harvests for UME-affected trees were not found to be a function of percent nut set or yield effi-

Table 1. Comparisons of trunk diameter, kernel yield, and yield efficiency between healthy and unpruned and pruned union mild etch (UME)–affected Mission almond trees[y]

	Trunk diameter (cm)				Kernel yields (kg per tree)				Yield efficiency (gm/cm^2)			
Tree type	1991	1992	1993	1994	1991	1992	1993	1994	1991	1992	1993	1994
Healthy	11.9 a	14.9 a	16.8 a	18.0 a	0.85 a	ND[z]	4.13 a	5.68 a	7.66 c	ND	18.68 a	22.36 a
Unpruned, UME-affected	8.8 b	11.7 b	13.7 b	14.8 c	0.62 b	ND	2.35 b	4.17 b	10.21 a	ND	16.03 b	24.29 a
Pruned, UME-affected	8.8 b	12.1 b	14.2 b	17.4 b	0.55 b	ND	2.54 b	4.42 b	9.08 b	ND	16.16 b	23.88 a

[y] Includes 17 healthy and 20 UME-affected trees. Pruning consisted of thinning primary limbs. Analysis of variance with PROC GLM (SAS Institute, Cary, NC) in unbalanced data sets is presented here. Within each column, means followed by the same letter are not significantly different, least significant difference, $P = 0.05$.

[z] Not done.

ciency (in 1994 both components were similar statistically for diseased and healthy trees), but they apparently resulted from smaller tree size and less fruiting capacity.

UME differs in symptomatology from almond brown line decline (ABLD), another union disorder of almond/Marianna 2624 trees. In contrast with trees that exhibit UME, ABLD-affected trees produce severely stunted shoots and a prominent line of necrotic bark tissue along the graft union accompanied by deep pits in the woody cylinder (see Figure 1), and trees decline rapidly. Furthermore, symptoms of ABLD are induced by infection with peach yellow leafroll phytoplasma (5).

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